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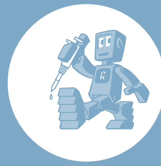

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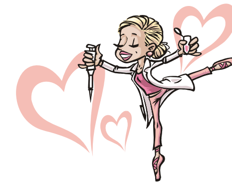
NG dART RT KIT

– for reverse transcription assays –



NG dART RT Kit
for Reverse Transcription

Cat. No.	Size
E0801-01	25 reactions
E0801-03	50 reactions
E0801-02	100 reactions

Storage Conditions:

Store at -20°C

Component	25 Rxn Kit	50 Rxn Kit	100 Rxn Kit
NG dART RT Mix	25 µl	50 µl	100 µl
5 x NG cDNA buffer	100 µl	200 µl	400 µl
Oligo(dT)$_{20}$ (50 µM)	25 µl	50 µl	100 µl
Random hexamers (200 ng/µl)	25 µl	50 µl	100 µl
RNase free water	1 x 1.0 ml	2 x 1.0 ml	4 x 1.0 ml

Two-Step RT-PCR: Protocol Overview:

First step (this kit): cDNA synthesis starts from either total RNA or from poly(A)$^+$-RNA. Primers are oligo(dT), random hexamers or reverse (anti-sense) gene specific primers.

Second step: Aliquots of the generated cDNA serve as template for PCR reactions in separate reaction tubes. Specific primer pairs are used for dsDNA amplification. Recommended enzyme preparations for optical, quantitative PCR methods are SG qPCR Master Mix (Cat.No. E0401, E0411), Probe Master Mix (E0420, E0422) or HotStart Perpetual Taq DNA Polymerase (E2700). For subsequent molecular cloning or for sequencing of amplified cDNA, we recommend using OptiTaq DNA polymerase (E2600) or OptiTaq Master Mix (E2910).

Quality Control:

All preparations are assayed for contaminating endonuclease and exonuclease and nonspecific RNase and single- and double-stranded DNase activities.

The dART kit is optimized for high sensitivity RT reactions. It enables full length cDNA synthesis with high yield, even from rare, low copy number or delicate RNA templates. Featuring a carefully optimized, modified Reverse Transcriptase, the dART kit performs highly specific RT reactions.

Description:

→ For cDNA synthesis and for two-step RT-PCR reactions requiring high sensitivity and high specificity.

→ NG dART enzyme mix contains dART reverse transcriptase with improved thermostability (up to 65°C) and an RNase inhibitor, offering protection from nucleolytic attack by RNases A, B, or C, respectively. The optimized 5x NG cDNA buffer contains dNTPs.

→ Synthesizes single-stranded DNA from RNA templates in a broad range of temperatures between 35°C and 65°C.

→ High cDNA yield and full-length reverse transcripts for RNA templates with a wide range of G+C content.

→ No detectable RNase activity for single-stranded RNA. Reduced RNase H activity (DNA/RNA hybrid molecules only). Since RT reactions are non-amplifying reactions, RNase H activity generally has no influence on cDNA product length and product yield.

→ Suitable for preparation of labeled hybridization probes.

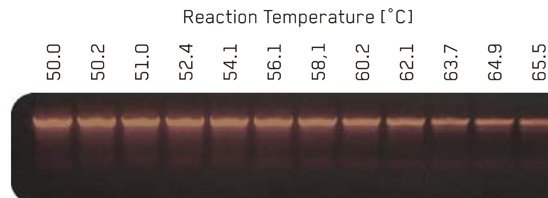


Fig. 1: Temperature gradient RT-PCR using NG dART Reverse Transcriptase. mRNA for *Sus scrofa* arginase I gene fragment was reverse transcribed with NG dART reverse transcriptase. RT was started from total RNA, isolated from pig liver using the GeneMATRIX Universal RNA Purification Kit (EURx Cat. No. E3598). PCR amplification with OptiTaq DNA Polymerase (E2600). Amplicon length: 1137 bp.

dART Reaction Protocol:

1. Thaw 5x cDNA buffer, vortex gently and allow enough time for equilibration to room temperature. Any remaining white precipitate should dissolve completely. Buffer is ready to use, when appearing completely clear without any visible turbidity.

2. Assemble cDNA synthesis assay in a RNase free plastic reaction tube:

Component	**Amount**
5x NG cDNA buffer	4 µl
Primer*	1 µl
RNA (10 ng-5 µg)	x µl
NG dART RT Mix	1 µl
RNase free water	@ 20 µl

Total volume: 20 µl.

* Use 50 µM Oligo(dT)$_{20}$, 200 ng/µl random hexamer primer, or 10 µM reverse gene specific primer, respectively.

3. Preheat a thermal cycler to an appropriate temperature (see below). Transfer the assembled reaction to the preheated cycler.

Oligo(dT)$_{20}$ primed	30-60 min @ 50°C (or 35-65°C)
Gene specific primed	30-60 min @ 50°C (or 35-65°C)
Random hexamer primed	10 min @ 25°C, followed by 20-50 min @ 50°C (or 35-65°C)

4. Terminate the reaction by incubating at 85°C for 5 min.

5. cDNA is ready for immediate use e.g. in PCR. Use 2-5 µl cDNA per 50 µl PCR reaction. Alternately, store cDNA at -20°C .